Outcome Summary

	ANC500 & median time	Plt 20 K % median tim	Grade II-IV aGvHD	Extensive cGvHD	1-Yr TRM	1-Yr OS	1-Yr DFS
All Patients (n = 87)	87 ± 7% 20 days	77 ± 7% 50 days	34 ± 6%	12 ± 4%	19 ± 4%	81 ± 4%	68 ± 6%
NW (n = 57)	92 ± 9% 19 days	85 ± 9% 46 days	37 ± 8%	3 ± 3%	10 ± 4%	90 ± 4%	78 ± 6%
W (n = 30)	80 ± 11% 22 days	65 ± 11% 58 days	31 ± 8%	23 ± 8%	35 ± 9%	65 ± 9%	53 ± 10%
Cox RR & P-value (W Reference)	1.76 0.03	1.67 0.07	1.20 0.65	0.11 0.04	0.27 0.02	0.27 0.02 (death)	0.54 0.13 (death or relapse)

SOLID TUMORS

71

ADOPTIVE INFUSION OF DONOR NK CELLS REDUCES GVHD IN RECIPIENTS OF T-CELL DEPLETED AND T-CELL REPLETE ALLOGENEIC MHC-MATCHED HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Retrospective data suggest that alloreactive NK cell-mediated reduction in GVHD and disease relapse may be limited to patients with AML undergoing T-cell depleted KIR ligand-mismatched allogeneic hematopoietic cell transplantation (HCT). Whether NK cells can mediate similar beneficial effects following MHC-matched, T-cell replete allogeneic HCT is currently unknown. We investigated if an infusion of purified donor NK cells would reduce GVHD and simultaneously mediate anti-tumor effects in a murine solid tumor model of MHC-matched allogeneic HCT. Ten days following intravenous injection of 10^5 RENCA tumor cells, BALB/c recipient mice were irradiated (950 cGy) and transplanted with 8×10^6 bone marrow cells from MHC-matched B10.d2 donors. Recipients of a T-cell replete HCT additionally received 15×10^6 splenocytes from MHC-matched B10.d2 donors on day 0 whereas recipients of a T-cell depleted HCT received no splenocytes on day 0 with or without a delayed infusion of donor lymphocytes (15×10^6 splenocytes) on day +4. All mice in groups receiving splenocytes (day 0 or day +4) developed GVHD (weight loss, alopecia and hunched posture) by day +30 and died before day +60. In contrast, 60% of mice receiving splenocytes and purified donor NK cells ($0.5–5 \times 10^6$) on day 0 or day +4 remained free of skin GVHD and had significantly lower GVHD scores (see Table). Moreover, 40% of transplant recipients receiving splenocytes and NK cells on either day 0 or day +4 survived ≥60 days post transplant. To further characterize the NK cell subset responsible for prolonging GVHD-free survival, donor NK cells were sorted into BALB/c non-alloreactive Ly49 ligand-matched (Ly49G2) and BALB/c alloreactive Ly49 ligand-mismatched (Ly49C) NK cells. Recipients of Ly49C+ NK cells (given day +5) had significantly less GVHD and prolonged survival ($p < 0.01$) compared to HCT recipients receiving Ly49G2+ NK cells. No difference in GVHD-free survival was observed between transplant recipients of Ly49G2+ NK cells and controls not receiving NK cells. Pulmonary tumor burden was significantly ($p < 0.01$) lower in recipients of Ly49C+ or Ly49G2+ NK cells compared to mice not receiving NK cells. These data provide in vivo evidence that a single infusion of alloreactive donor NK cells can prolong survival by both reducing GVHD and mediating anti-tumor effects following MHC-matched T-cell replete allogeneic HCT.

Table. *GVHD scores between day +25 and +45 in 4 different cohorts of HCT recipients*

Groups	#1 (T-R)	#2 (T-D)	#3 (T-R)	#4 (T-D)
Transplant type	splenocytes (d0) (no NK cells)	splenocytes (d + 4) (no NK cells)	splenocytes (d0) + NK (d + 4)	splenocytes (d + 4) + NK (d0)
GVHD score ± SD	2.8 ± 0.4	3.0 ± 0.7	0.5 ± 0.4	0.7 ± 0.2

T-R = T cell replete HCT, T-D = T cell deplete HCT. Groups #3 or #4 vs. #1 or #2 = $p < 0.001$, students unpaired T-test.

STEM CELL BIOLOGY

72

RECIPIENT BASED STRATEGIES FOR IMPROVED TRANSPLANT EFFICIENCY: CD26 INHIBITOR TREATED AND CD26$^{-/-}$ RECIPIENT MICE EXHIBIT INCREASED SHORT-TERM HOMING AND LONG-TERM ENGRAFTMENT OF HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Introduction: Through the use of CD26 inhibitors and CD26 deficient mice (CD26$^{-/-}$), we have previously generated data suggesting that suppression of CD26/DPPIV activity on the transplant donor cell population could potentially be utilized clinically as a method of increasing transplant efficiency. However, the clinical importance of the transplant recipient should not to be overlooked. We therefore investigated whether inhibition or loss of CD26 activity in the recipient would have an effect on hematopoietic stem cell transplantation utilizing an *in vivo* congenic mouse model of transplantation. **Methods:** The short-term homing and long-term engraftment of BoyJ donor cells (expressing CD45.1$^+$) into lethally irradiated control C57BL/6, CD26 inhibitor (Diprotin A) treated C57BL/6, or CD26$^{-/-}$ mice (expressing CD45.2$^+$) was monitored by flow cytometric analysis of the bone marrow and peripheral blood at 24 hours and 6 months post-transplant. **Results:** Twenty-four hours post-transplant of 20×10^6 BoyJ mononuclear cells, we observed 8.85 ± 0.58%, 10.69 ± 1.01%, and 12.45 ± 1.33% donor derived Sca-1$^+$lin$^-$ cells in the bone marrow of recipient mice for control, Diprotin A treated, and CD26$^{-/-}$ recipient mice respectively. As compared to control mice, this represents a 20.8% increase ($p = 0.01$) with CD26 inhibitor treatment and a 40.7% increase ($p \leq 0.05$) resulting from the use of a CD26$^{-/-}$ recipient in short-term homing ($N = 5$ mice per group). Six months post-transplant of 1×10^5 BoyJ mononuclear cells, we observed 39.90 ± 4.38%, 70.22 ± 3.72%, and 92.51 ± 1.04% donor contribution to hematopoiesis in the peripheral blood of control, Diprotin A treated, and CD26$^{-/-}$ recipient mice respectively. This represents a 76.0% increase ($p \leq 0.01$) with CD26 inhibitor treatment and a 131.9% increase ($p \leq 0.01$) as a result of the CD26$^{-/-}$ recipient in long-term engraftment as compared to control recipient mice ($N = 14$ mice per group). **Conclusions:** These results provide pre-clinical evidence of the importance of CD26 expression within the transplant recipient with regard to regulating hematopoietic stem cell homing and engraftment. Our results also support the potential use of CD26 inhibitors to treat transplant patients during hematopoietic stem cell transplantation as a method of improving transplant efficiency.

73

HUMAN HEMATOPOIETIC PROGENITORS WITH SLOW DIVISIONAL KINETICS RECONSTITUTE T CELLS IN THYMUS, B CELLS IN LYMPH NODES AND MYELOID CELLS IN MARROW OF NOD/SCID MOUSE MODEL AND REPRESENT THE MOST PRIMITIVE FRACTION

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We have demonstrated that CD34+ cells with slow division kinetics are associated with self renewal and increased frequency of LTC-IC and myeloid-lymphoid initiating cells (ML-IC) in vitro. The present study showed that only the slow dividing fraction (SDF) of human CD34+ cells, but not the fast dividing fraction (FDF), is able to engraft the thymus and lymph nodes of NOD/SCID mice.